

FIG. 1A

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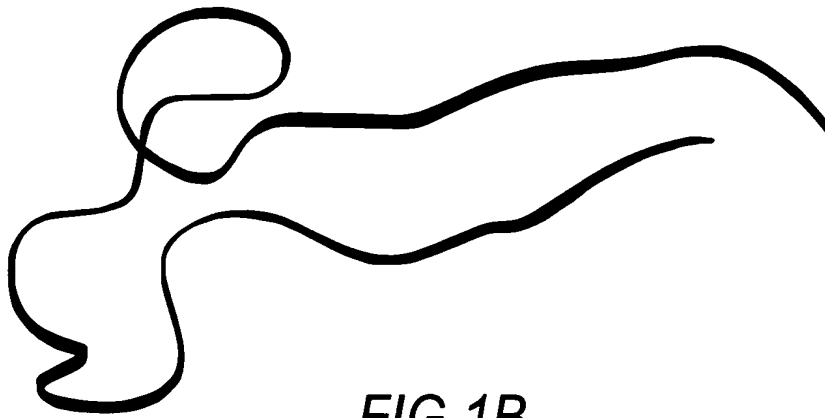


FIG.1B

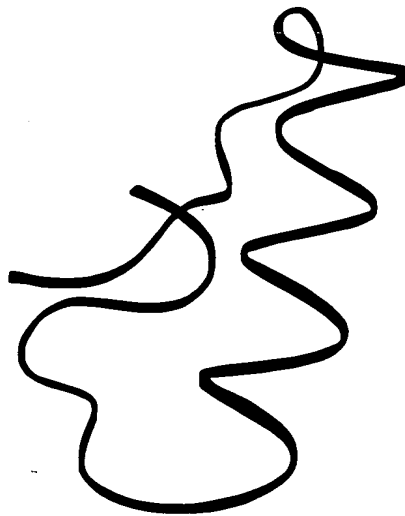


FIG.1C

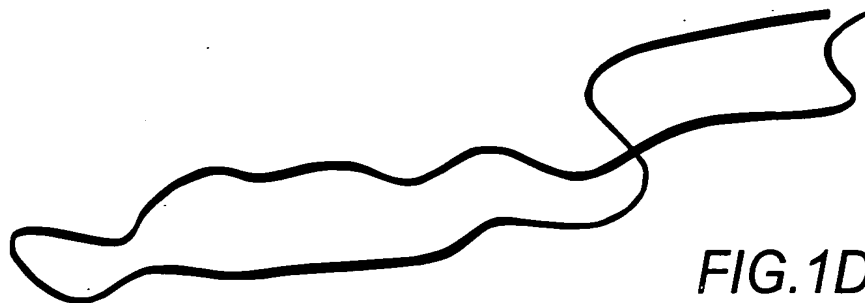
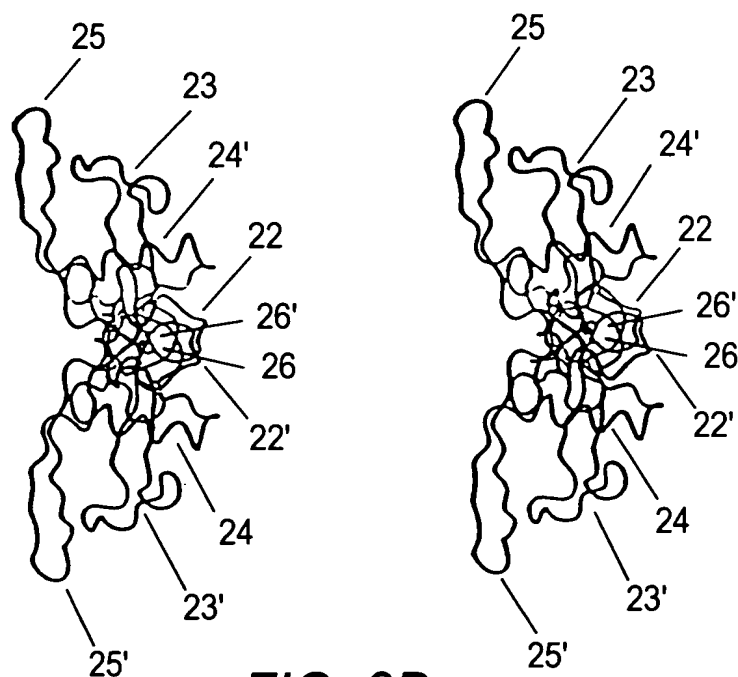
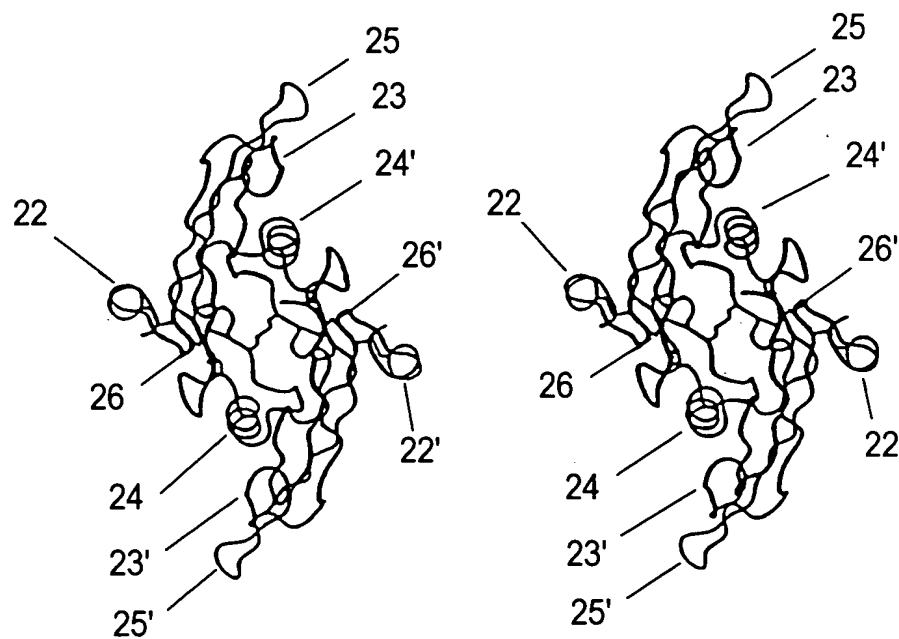


FIG.1D

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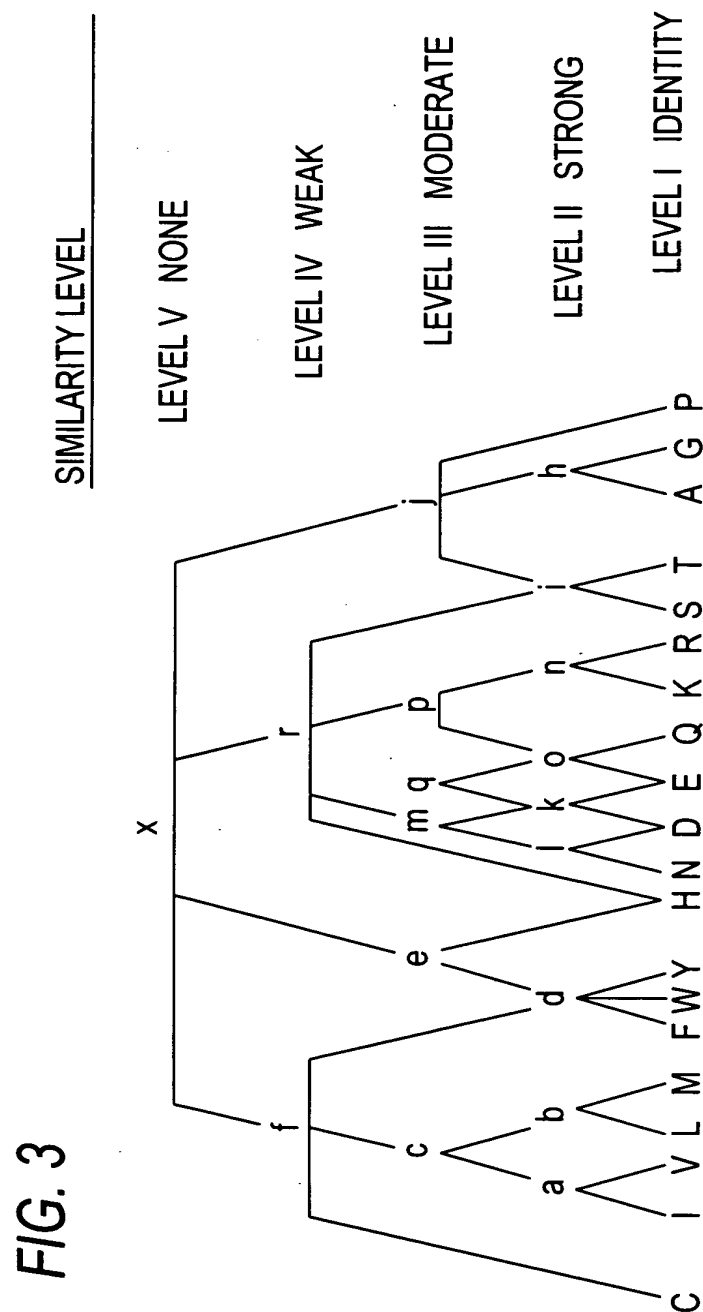
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Attorney: Karen Mangasarian (Reg. No. 43,772)

For: MODIFIED TGF- β SUPERFAMILY PROTEINS

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FIG. 4

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OP-1	CCA - - PTQLNAI SVLYFDDS- SNVI LKKYRNMVVRA	CGCH
BMP-5	CCA - - PTKLNAI SVLYFDDS- SNVI LKKYRNMVVRS	CGCH
BMP-6	CCA - - PTKLNAI SVLYFDDN- SNVI LKKYRNMVVRA	CGCH
OP-2	CCA - - PTKLSATSVLYYDSS- NNVI LRKHRNMVVKAC	CGCH
OP-3	CCV - - PTELSAI SLLYYDRN- NNVI LRRERNMVVQAC	CGCH
60A	CCA - - PTRLGALPVL YHLND- ENVNLKKYRNMI VKS	CGCH
Vg-1	CCV - - PTKMSPI SMLFYDNN- DNVVL RHYENMAVDE	CGCR
UNIVIN	CCA - - PTKLSGI SMLYFDNN- ENVVL RQYEDMVVEAC	CGCR
BMP-2	CCV - - PTELSAI SMLYLDEN- EKVVL KNYQDMVVEG	CGCR
BMP-4	CCV - - PTELSAI SMLYLDEY- DKVVL KNYQEMVVEG	CGCR
GDF-5	CCV - - PTRLSPI SILFIDSA- NNVVYKQYEDMVVES	CGCR
GDF-6	CCV - - PTKLTPI SILYIDAG- NNVVYKQYEDMVVES	CGCR
GDF-7	CCV - - PARLSPI SILYIDAA- NNVVYKQYEDMVVEAC	CGCR
CDMP-2	CCV - - PTKLTPI SILYIDAG- NNVVYNEYEEMVVES	CGCR
dpp	CCV - - PTQLDSVAMLYLNDQ- STVVL KNYQEMTVVG	CGCR
BMP-9	CCV - - PTKLSPI SVLYKDDMGVPTL KYHYEGMSVAE	CGCR
DORSALIN	CCV - - PTKLDAI SILYKDDAGVPTLI YNYEGMKVAE	CGCR
BMP-10	CCV - - PTKLEPI SILYLDKG- VVTYKFKYEGMAVSE	CGCR
GDF-3	VCCV - - PTKLSPI SMLYQDSD- KNVI LRHYEDMVVDE	CGCG
GDF-1	CCV - - PERLSPI SVLFFDNE- DNVVL RHYEDMVVDE	CGCR
SCREW	CCV - - PTVLGAI TILRYLNE- DIIDLTKYQKAVAKE	CGCH
BMP-3	CCV - - PEKMSSL SILFFDEN- KNVVLKVYPNMTVES	CACR
NODAL	CCA - - PVKTKPLSMLYVDN- - GRVLL EHHKDMI VEE	CGCL
TGF- α 2	CCV - - SQDLEPLTILYYIG- - KTPKIEQLSNMI VKS	CKCS
TGF- α 3	CCV - - PQDLEPLTILYYVG- - RTPKVEQLSNMVVKS	CKCS
TGF- α 4	CCV - - PQTLDPLPIIYYVG- - RNVRVEQLSNMVVRAC	CKCS
TGF- α 1	CCV - - PQALEPLPIVYYVG- - RKPKVEQLSNMI VRS	CKCS
TGF- α 5	CCV - - PDVLEPLPIIYYVG- - RTAKVEQLSNMVVRSC	CNCS
GDF-9	SICV - - PGKYSPLSVLTI EPD- GSIAYKEYEDMI ATR	CTCR
Inhibin α	CCAALPGTMRPLHVRTTSDGGYSFKYETVPNLLTQH	CACI
Inhibin β A	CCV - - PTKLRPMSMLYYDDG- QNI I KKDI QNMI VEE	CGCS
Inhibin β B	CCI - - PTKLSTMSMLYFDDE- YNI VKRDVPNMI VEE	CGCA
Inhibin β C	CCV - - PTARRPLSLLYYDRD- SNI VKTDI PDMVVEAC	CGCS
MIS	CCV - - PTATAGKLLI SLSE- - ERI SAHVPNMVATE	CGCR
GDNF	CCR - - PIAFDDD- - LSFLD- - DNLVYHI LRKHS	AKRCGCI
BMP-11	CCT - - PTKMSPINMLYFNDK- QQI I YGKI PGMVVDR	CGCS
GDF-9	SICV - - PGKYSPLSVLTI EPD- GSIAYKEYEDMI ATR	CTCR

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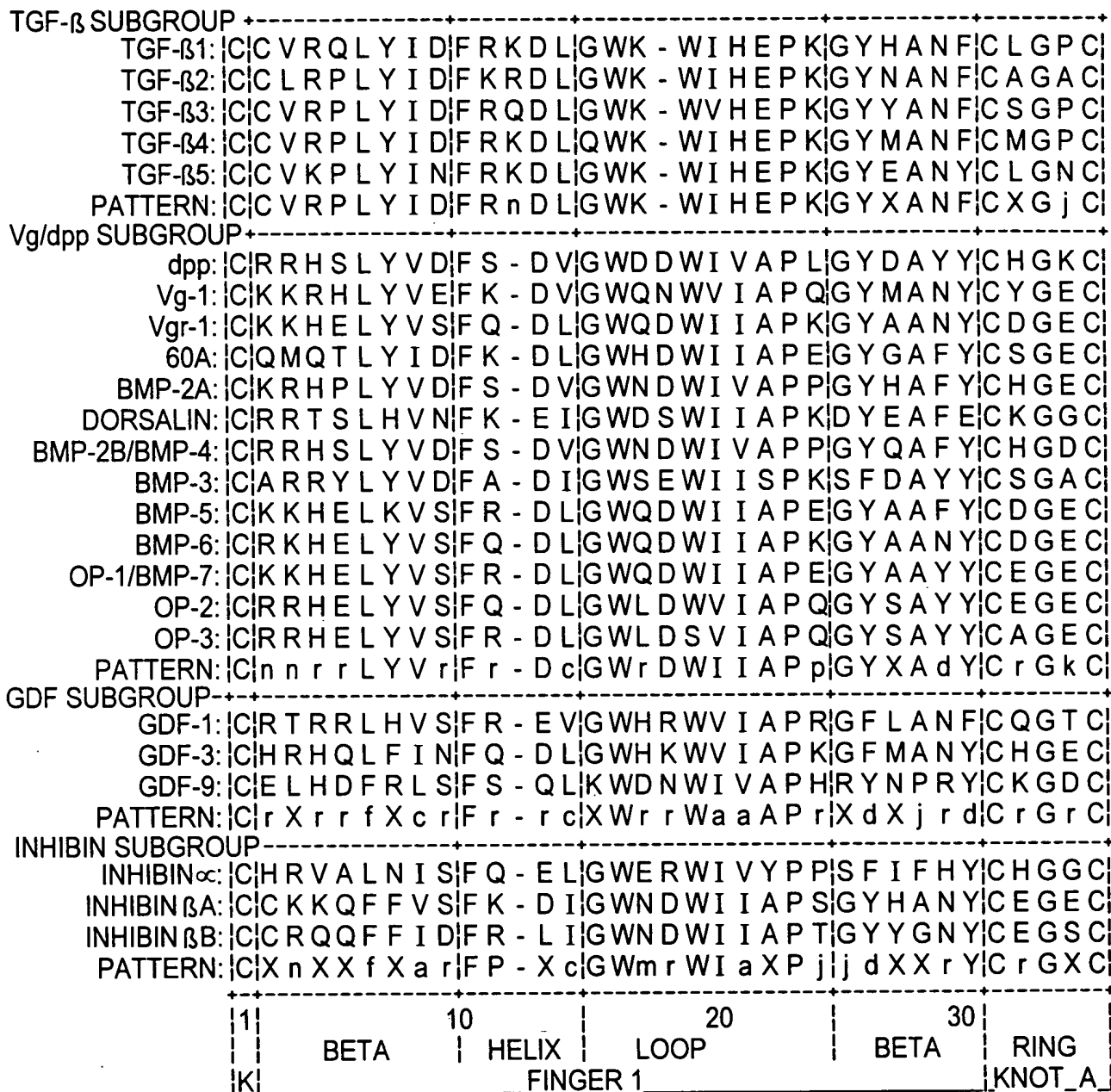


FIG. 5A

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TGF- β SUBGROUP																																									
TGF- β 1:	P	Y	I	W	S	-	-	-	-	-	L	D	T	Q	Y	S	K	V	L	A	L	Y	N	Q	H	N	P	-	-	G	A	S	A	A	P		C		C		
TGF- β 2:	P	Y	L	W	S	-	-	-	-	-	S	D	T	Q	H	S	R	V	L	S	L	Y	N	T	I	N	P	-	-	E	A	S	A	S	P		C		C		
TGF- β 3:	P	Y	L	R	S	-	-	-	-	-	A	D	T	T	H	S	T	V	L	G	L	Y	N	T	L	N	P	-	-	E	A	S	A	S	P		C		C		
TGF- β 4:	P	Y	I	W	S	-	-	-	-	-	A	D	T	Q	Y	T	K	V	L	A	L	Y	N	Q	H	N	P	-	-	G	A	S	A	A	P		C		C		
TGF- β 5:	P	Y	I	W	S	-	-	-	-	-	M	D	T	Q	Y	S	K	V	L	S	L	Y	N	Q	N	N	P	-	-	G	A	S	I	S	P		C		C		
PATTERN:	P	Y	c	W	S	-	-	-	-	-	X	D	T	Q	e	S	n	V	L	j	L	Y	N	r	X	N	P	-	-	X	A	S	A	j	P		C		C		
Vg/dpp SUBGROUP																																									
dpp:	P	F	P	L	A	D	H	F	-	-	-	N	S	T	N	H	A	V	V	Q	T	L	V	N	N	M	N	P	-	-	G	K	V	P	K	A		C		C	
Vg-1:	P	Y	P	L	T	E	I	L	-	-	-	N	G	S	N	H	A	I	L	Q	T	L	V	H	S	I	E	P	-	-	E	D	I	P	L	P		C		C	
Vgr-1:	S	F	P	L	N	A	H	M	-	-	-	N	A	T	N	H	A	I	V	Q	T	L	V	H	L	M	N	P	-	-	E	Y	V	P	K	P		C		C	
60A:	N	F	P	L	N	A	H	M	-	-	-	N	A	T	N	H	A	I	V	Q	T	L	V	H	L	L	E	P	-	-	K	K	V	P	K	P		C		C	
BMP-2A:	P	F	P	L	A	D	H	L	-	-	-	N	S	T	N	H	A	I	V	Q	T	L	V	N	S	V	N	-	-	-	S	K	I	P	K	A		C		C	
DORSALIN:	F	F	P	L	T	D	N	V	-	-	-	T	P	T	K	H	A	I	V	Q	T	L	V	H	L	Q	N	P	-	-	K	K	A	S	K	A		C		C	
BMP-2B/BMP-4:	P	F	P	L	A	D	H	L	-	-	-	N	S	T	N	H	A	I	V	Q	T	L	V	N	S	V	N	-	-	-	S	S	I	P	K	A		C		C	
BMP-3:	Q	F	P	M	P	K	S	L	-	-	-	K	P	S	N	H	A	T	I	Q	S	L	V	R	A	V	G	V	V	-	P	G	I	P	E	P		C		C	
BMP-5:	S	F	P	L	N	A	H	M	-	-	-	N	A	T	N	H	A	I	V	Q	T	L	V	H	L	M	F	P	-	-	D	H	V	P	K	P		C		C	
BMP-6:	S	F	P	L	N	A	H	M	-	-	-	N	A	T	N	H	A	I	V	Q	T	L	V	H	L	M	N	P	-	-	E	Y	V	P	K	P		C		C	
OP-1/BMP-7:	A	F	P	L	N	S	Y	M	-	-	-	N	A	T	N	H	A	I	V	Q	T	L	V	H	F	I	N	P	-	-	E	T	V	P	K	P		C		C	
OP-2:	S	F	P	L	D	S	C	M	-	-	-	N	A	T	N	H	A	I	L	Q	S	L	V	H	L	M	K	P	-	-	N	A	V	P	K	A		C		C	
OP-3:	I	Y	P	L	N	S	C	M	-	-	-	N	S	T	N	H	A	T	M	Q	A	L	V	H	L	M	K	P	-	-	D	I	I	P	K	V		C		C	
PATTERN:	X	F	P	L	X	X	X	b	-	-	-	N	j	T	N	H	A	I	a	Q	T	L	V	r	X	c	r	z	z	-	r	X	a	P	K	j		C		C	
GDF SUBGROUP																																									
GDF-1:	A	L	P	E	T	L	R	G	P	G	G	P	P	A	L	N	H	A	V	L	R	A	L	M	H	A	A	A	P	T	-	P	G	A	G	S	P		C		C
GDF-3:	P	F	S	M	T	T	Y	L	-	-	-	N	S	S	N	Y	A	F	M	Q	A	L	M	H	M	A	D	-	-	-	P	K	V	P	K	A		V		C	
GDF-9:	P	R	A	V	R	H	R	Y	-	-	-	G	S	P	V	H	T	M	V	Q	N	I	I	Y	E	K	L	D	-	-	P	S	V	P	R	P		S		C	
PATTERN:	j	X	j	X	r	X	X	X	z	z	z	X	j	X	X	e	j	f	c	p	X	c	c	e	X	X	X	z	z	-	P	X	X	j	r	j		X		C	
INHIBIN SUBGROUP																																									
INHIBIN α :	G	L	H	I	P	P	N	L	S	L	-	-	P	V	P	G	A	P	P	T	P	A	Q	P	Y	S	L	-	-	-	L	P	G	A	Q	P		C		C	
INHIBIN β A:	P	S	H	I	A	G	T	S	G	S	-	-	S	L	S	F	H	S	T	V	I	N	H	Y	R	M	R	G	H	S	P	F	A	N	L	K	S		C		C
INHIBIN β B:	P	A	Y	L	A	G	V	P	G	S	-	-	A	S	S	F	H	T	A	V	V	N	Q	Y	R	M	R	G	L	N	-	P	G	T	V	N	S		C		C
PATTERN:	j	X	e	c	j	j	X	X	j	X	-	-	j	X	j	X	X	j	j	X	X	X	r	X	X	X	X	z	z	z	z	X	j	X	X	r	j		C		C

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TGF- β SUBGROUP															
TGF- β 1:	V - - PQA	LEPLPIVY	YVG - - RKP KVEQLSNMIVRS CKC S												
TGF- β 2:	V - - SQD	LEPLTILY	YIG - - KTP KIEQLSNMIVKS CKC S												
TGF- β 3:	V - - PQD	LEPLTILY	YVG - - RTP KVEQLSNMVVK S CKC S												
TGF- β 4:	V - - PQT	LDPLPIIY	YVG - - RNV RVEQLSNMVVRA CKC S												
TGF- β 5:	V - - PDV	LEPLPIIY	YVG - - RTA KVEQLSNMVVRS CNC S												
PATTERN:	V - - PQX	LEPLjIcY	YVG - - Rrj KVEQLSNMaVnS CKC S												
Vg/dpp SUBGROUP															
dpp:	V - - PTQ	LDSVAMLY	LNDQ - STV VLKNYQEMTVVG CGC R												
Vg-1:	V - - PTK	MSPISMLF	YDNN - DNV VLRHYENMAVDE CGC R												
Vgr-1:	A - - PTK	LNAISVLY	FDDN - SNV ILKKYRNMVVRA CGC H												
60A:	A - - PTR	LGA LPVLY	HLND - ENV NLKKYRNMIVKS CGC H												
BMP-2A:	V - - PTE	LSAISMLY	LDEN - EKV VLKNYQDMVVEG CGC R												
DORSALIN:	V - - PTK	LDAISILY	KDDAGVPT LIYNYEGMKVAE CGC R												
BMP-2B/BMP-4:	V - - PTE	LSAISMLY	LDEY - DKV VLKNYQEMVVEG CGC R												
BMP-3:	V - - PEK	MSSLSILF	FDEN - KNV VLKVYPNMTVES CAC R												
BMP-5:	A - - PTK	LNAISVLY	FDDS - SNV ILKKYRNMVVRS CGC H												
BMP-6:	A - - PTK	LNAISVLY	FDDN - SNV ILKKYRNMVVRA CGC H												
OP-1/BMP-7:	A - - PTQ	LNAISVLY	FDDS - SNV ILKKYRNMVVRA CGC H												
OP-2:	A - - PTK	LSATSVLY	YDSS - NNV ILRKHNRNMVVK A CGC H												
OP-3:	V - - PTE	LSAISLLY	YDRN - NNV ILRRERNMVVQA CGC H												
PATTERN:	X - - PT	pLrAaScLY	fDmrzrrV aLn rYpIMXVp j CGC R												
GDF SUBGROUP															
GDF-1:	V - - PER	LSPISVLF	FDNS - DNV VLRHYEDMVVDE CGC R												
GDF-3:	V - - PTK	LSPISMLY	QDSD - KNV ILRHYEDMVVDE CGC G												
GDF-9:	V - - PGK	YSPLSVLT	IEPD - GSI AYKEYEDMIATRC TC R												
PATTERN:	V - - PX	n fSPcScLX	XkXr - Xra Xfn rYEDMaXrp CjC X												
INHIBIN SUBGROUP															
INHIBIN α :	A A L	P G T M R P L H V R T	T S D G G Y S F K Y E T V P N L L T Q H C A C I												
INHIBIN β A:	V - - PTK	L R P M S M L Y	Y D D G - Q N I I K K D I Q N M I V E E C G C S												
INHIBIN β B:	I - - PTK	L S T M S M L Y	F D D E - Y N I V K R D V P N M I V E E C G C A												
PATTERN:	X z z P j r b r j b r c X X	X r D X z X r f	X X p r a X N b c X o r C h C X												
<table border="1"> <tr> <td>80</td><td>90</td><td>100</td><td>110</td></tr> <tr> <td>BETA</td><td>LOOP</td><td>BETA</td><td>RING</td></tr> <tr> <td colspan="3">FINGER_2</td><td>KNOT2C</td></tr> </table>				80	90	100	110	BETA	LOOP	BETA	RING	FINGER_2			KNOT2C
80	90	100	110												
BETA	LOOP	BETA	RING												
FINGER_2			KNOT2C												

FIG. 5C

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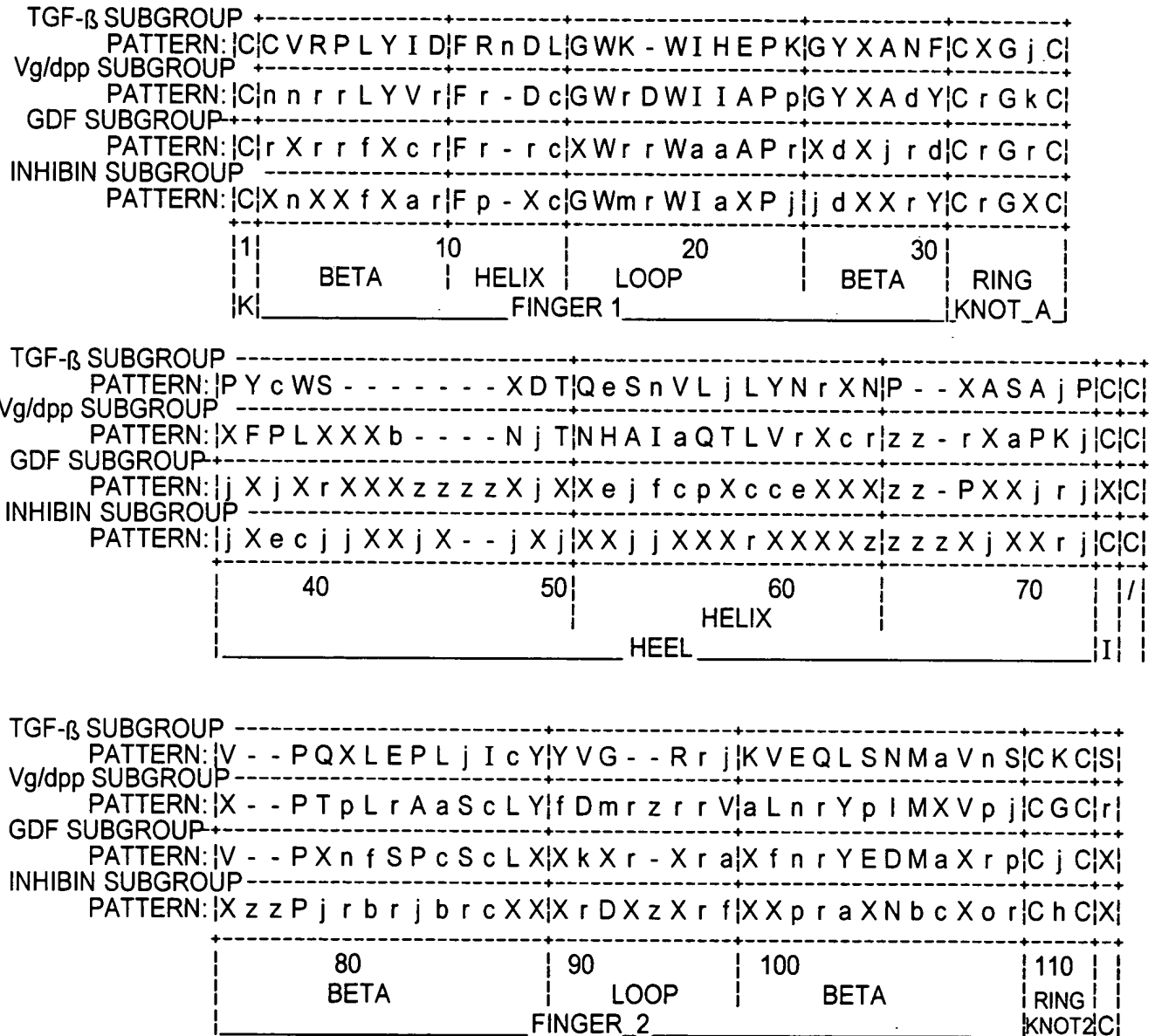


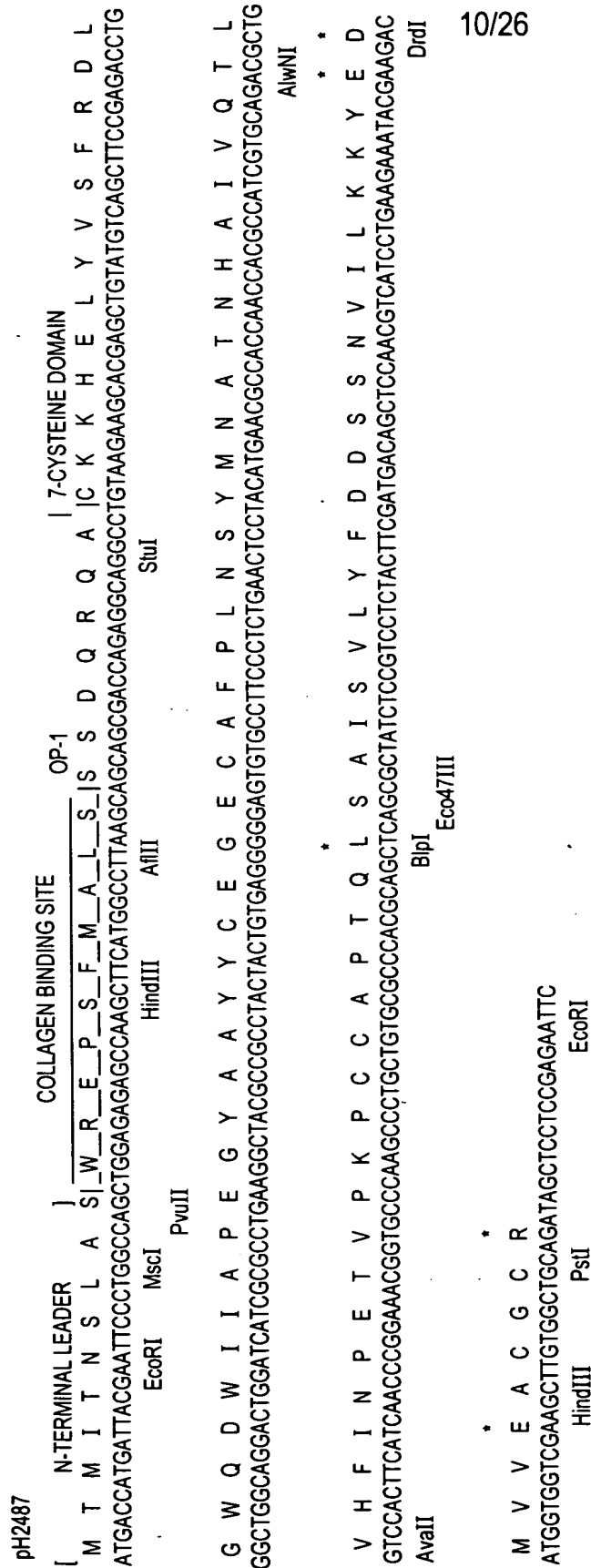
FIG. 6

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FIG. 7A

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pH2440 His-6 ATTACHED AT 35 RESIDUES UPSTREAM OF FIRST CYSTEINE; POOR ACTIVITY!?

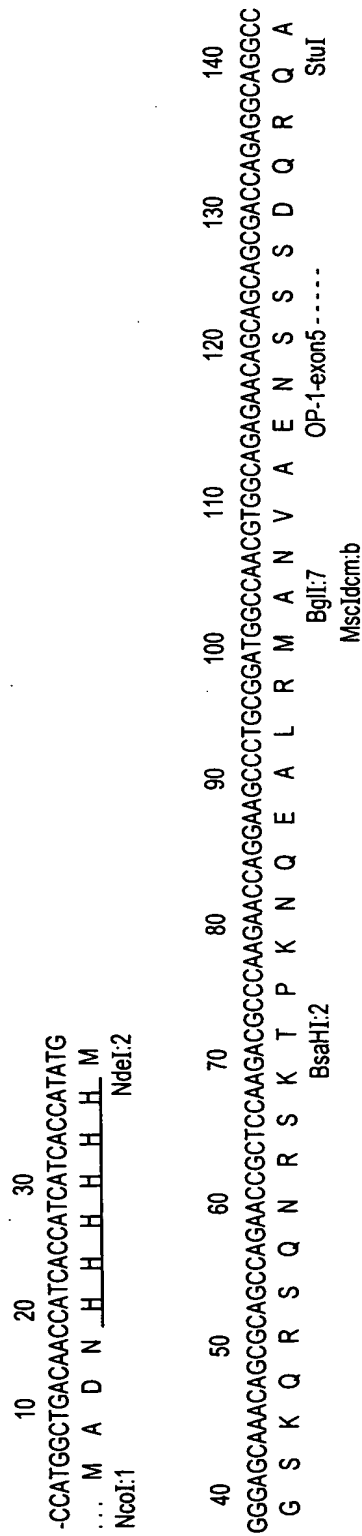


FIG. 7B

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pH2521 FB LEADER, AND 15 RESIDUES UPSTREAM FROM FIRST CYSTEINE

10 20 30 40 50 60 70 80 90 100 110 120
ATGATCGAATTCATGGCTGACAAACAAATTCACAAAGGAACAGACGAGCGGTTCTACGAGATCTTGCACCTGCCGAACTGAACGAAAGAGCAGCGCTTAACGGCTTCATCCAAAGCCTGAAA
M I E F M A D N K F N K E Q Q N A F Y E I L H L P N L N E E Q R N G F I Q S L K

EcoRI:1

MluI:1

BglII:1

XmnI:b

130 140 150 160 170 180 190
GAAGAGCCGCTCAGCTGCGAATCTGCTAGCGGATGCCAAGAACTGAACGATGCCGAGGCACCGAAATCGGCC
E E P S Q S A N L L A D A K K L N D A Q A P K S A

NheI:1

FspI:b

300 310 320 330 340
ATGGCAACGTGGCAGAGAACAGCAGCAGCGACCGACCCAGAGGCAGGCCT
M A N V A E N S S S D Q R Q A

BglI:7

op-1-exon5-----

StuI

NcoI:1

XcmI:8

MscIdcm:b

FIG. 7C

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pH2525 FB- AND His6-LEADER, RETAINING 35 RESIDUES UPSTREAM FROM FIRST CYSTEINE; GOOD REFOLDING

ATGATCGAAATTCATGGCTGACAAACAAATTCAACAGGAACAGCAGACGCGTTCTACGAGATCTTGCACTGCCGAACCTGAACGAAGAGCAGCGTAACGGCTTCATCCAAAGCCTGAAA

M I E F M A D N K F N K E Q Q N A F Y E I L H L P N L N E E Q R N G F I Q S L K

EcoRI:1

MluI:1

BglII:1

XmnI:b

GAAGAGCCGTCTCAGTGTGCGAATCTGCTAGCGGATGCCAAGAACTGAACGATGCCGAGGCCACCGAAATCGGCCATGGCTGACAAACCATCACCATCATCACCATATG

E E P S Q S A N L L A D A K K L N D A Q A P K S A M A D N H H H H H H H M

NheI:1

FspI:b

NcoI:1

NdeI:2

GGGAGCAAACAGCGCAGCCAGAACCGCTCCAAGACGCCCCAAGAACCCAGGAGCCCTGCGGATGGCCACGTTGGCAGAGAACAGCAGCAGCGACCGAGAGGCGCCT

G S K Q R S Q N R S K T P K N Q E A L R M A N V A E N S S S D Q R Q A

MscI

StuI

FIG. 7D

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pH2527 FB-His-6-TRUNCATED OP-1 WITH ACID CLEAVAGE SITE

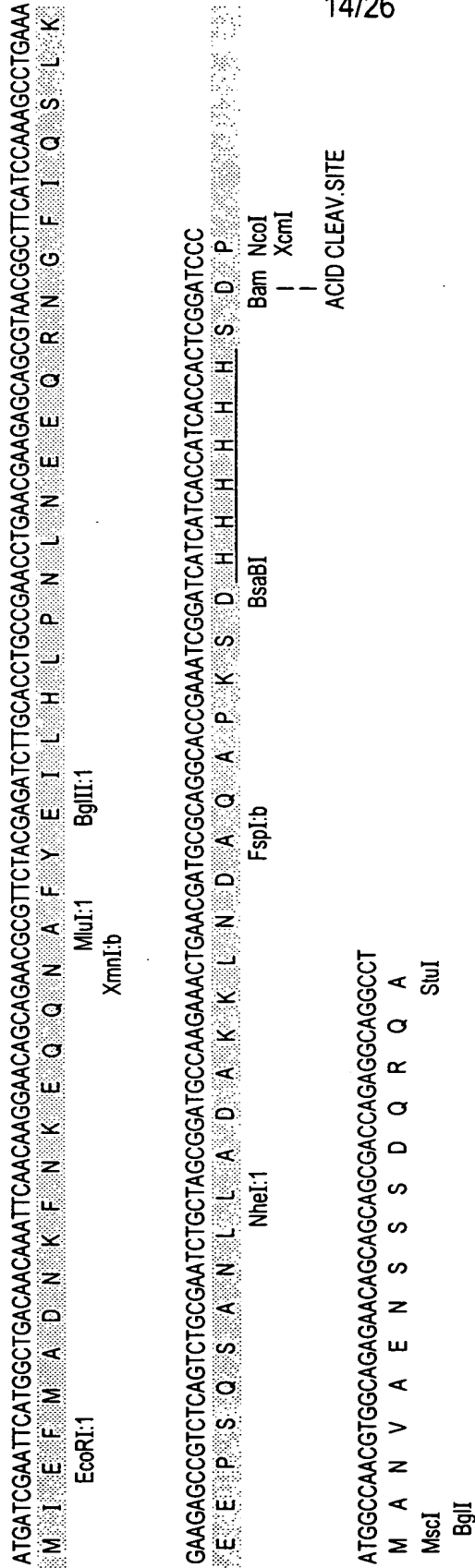


FIG. 7E

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H2528
FB-His6-CHIMP-3

```
10      20      30      40      50      60      70      80      90     100     110     120
-CCATGATCGAATTCATGGCTGACAAACAAATTCAACAAGGAACAGCAGAACGGTTCTACGAGATCTTGCACCTGCCGAACTGAACGAGCAGCGTAACGGCTTCATCCAAAGCCTG
... M I E F M A D N K F N K E Q Q N A F Y E I L H L P N L N E E Q R N G F I Q S L

130     140     150     160     170     180     190     200     210     220     230     240
AAAGAAAGCCGCTCAGTCTGCGAATCTGCTAGCGGATGCCAAGAACTGAACGATGCGCAGGCACCGAAATCGGATCATCATCACCATCACCACCTCGGATGCCGTTGGCCCGGG
K E E P S Q S A N L L A D A K K L N D A Q A P K S D H H H H H S D P M A L A G

250     260     270     280     290     300     310     320     330     340     350     360
ACGGTACAGCGCAGGGCAGCGGAGGTGCCGGCAGAGGTTCATGGTCGACGTGGTAGATCTCGCTGCAGCCGCAAGCCGTTGCACGTGGACTTCAAGGAGCTCGGCTGGGACGACTGG
T R T A Q G S G G A G R G H G R G R S R C S R K P L H V D F K E L G W D D W

370     380     390     400     410     420     430     440     450     460     470     480
ATCATCGCGCGCTGGACTACGAGGCGTACCACCTGCGAGGGGCTTTGCGACTTCCCTTTCGCGTTGCGACCTCGAGCCCCACCAACCATGCCATCATTCAGACGCTGCTCAACTCCATGGCA
I I A P L D Y E A Y H C E G L C D F P L R S H L E P T N H A I I Q T L L N S M A

490     500     510     520     530     540     550     560     570     580     590     600
CCAGACGCGCGCGCGCTCCTGCTGTGTGCCAGCGCGCTCAGCCCCCATCAGCATCCTTACATCGACGCGCGCCCAACAACGTTGTCTACAAGCAATACGAGGACATGGTGGTGGAGGCC
P D A A P A S C C V P A R L S P I S I L Y I D A A N N V V Y K Q Y E D M V V E A

610     620     630     640     650
TGGCGCTGTAGGTAAGCTTGTGGCTGCAGATAGCTCCTCCGAGAATTC
C G C R *
```

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FIG. 7F

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pH2469 TRUNCATED, GOOD ROS ACTIVITY; 14 ORIGINAL RESIDUES UPSTREAM OF FIRST CYSTEINE

	10	20	30	40	
-CCATGGCCCAACGTGGCAGAGAACACAGCAGCAGCGACCGACCGAGAGGGCAGGCC					
... M A N V A E N S S S D Q R Q A					
NcoI BglI:7			OP-1-exon5-----		StuI
MscIdcm:b					

FIG. 7G

pH2510 COLLAGEN SITE INSERTED 7 RESIDUES UPSTREAM OF CYSTEINE; GOOD EXPRESSION, REFOLDING

10 20 30 40 50 60 70 80 90 100 110
ATGTCCACGGGGAGCAACAGCGCAGCGCAAGACCGCTCCAAGACGCCCAAGAACCAAGAGCCCTGCGGATGGCCAGCTTCAATGGCCTTAAGCAGC
M S T G S K Q R S Q N R S K T P K N Q E A L R M A S W R E P S F M A L S S
BsaHI:2
BpmI+
MscIdcm:b
AflII:1
BfrI:1
PvuII

FIG. 7H

120 130
AGCGACCAGAGCGAGGCC
S D Q R Q A
StuI

pH2523 COLLAGEN PEPTIDE, AND SPACER ADDED AT 13 RESIDUES UPSTREAM FROM 1ST CYSTEINE

10 20 30 40 50 60 70 80 90 100 110
ATGTCCACGGGGAGCAACAGCGCAGCGCAAGACCGCTCCAAGACGCCCAAGAACCAAGAGCCCTGCGGATGGCCAGCTTCAATGGCCTTAAGCAGCAGCGAC
M S T G S K Q R S Q N R S K T P K N Q E A L R M A S W R E P S F M A L S S D
BsaHI:2
BpmI+
MscIdcm:b
AflII:1
BfrI:1
PvuII:b
DUPLICATION

FIG. 7I

120 130 140 150 160
CAGAGGAGGCCCAACGTGGCAGAGAACAGCAGCAGCGCAGCAGCGAGGCGAGCGCC
Q R Q A N V A E N S S S D Q R Q A
BglI OP-1-exon5-----
StuI

pH2524 Hexa-His, COLLAGEN PEPTIDE, SPACER ADDED AT 13 RESIDUES UPSTREAM FROM 1ST CYSTEINE

10 20 30
-CCATGGCTGACAACCATCACCATCATCACCATATG
... M A D N H H H H H M
NcoI:1
40 50 60 70 80 90 100 110 120 130 140
GGGAGCAACAGCGCAGCGCAAGACCGCTCCAAGACGCCCAAGAACCAAGAGCCCTGCGGATGGCCAGCTTCAATGGCCTTAAGCAGCAGCGACCGCAG
G S K Q R S Q N R S K T P K N Q E A L R M A S W R E P S F M A L S S D Q
BsaHI:2
BpmI+
MscIdcm:b
AflII:1
BfrI:1
PvuII:b
DUPLICATION

FIG. 7J

150 160 170
AGGCAGGCCCAACGTGGCAGAGAACAGCAGCAGCGCAGCAGCGAGGCGAGGCC
R Q A N V A E N S S S D Q R Q A
BglI OP-1-exon5-----
StuI

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7-CYSTEINE DOMAIN OF OP-1

FINGER-1

TGTAAGAAGCACGAGCTGTATGTCAGCTTCCGAGACCTGGGCTGGCAGGACTGGATCATCGCGCTGAAGGCTACGCCGCCCTACTACTGTGAGGGG
C K K H E L Y V S F R D L G W Q D W I I A P E G Y A A Y Y C E G

HEEL

GAGTGTGCCCTTCCCTCTGAACCTCTACATGAACGCCCAACCAACCGCCATCGTGCAGACGCTGGTCCACTTCATCAACCCGGAAACGGTGCCCAAGCCCTGC
E C A F P L N S Y M N A T N H A I V Q T L V H F I N P E T V P K P C

FINGER-2

TGTGCGCCACGACGCTCAATGCCATCTCCGTCCTCTACTTCGATGACAGCTCCAACGTCATCCTGAAGAAATACAGAAACATGGTGGTCCGGGCCCTGTGGCTGCCAC
C A P T Q L N A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C H

FIG. 8

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OP-1 CHIMERICS WITH CDMP-2 OR WITH BMP-2

PARENTAL MOLECULES: REFOLDING ACTIVITY (CELL BASED)

	FINGER1	HEEL	FINGER2			
OP-1				h	(-)	+++ (*)
BMP-2				r	+++	+++
CDMP-2				r	++++	+/-

REPLACING FINGER-1 OR HEEL:

H2383				r	+/-	N/A
H2362				r	+	N/A
H2360				r	+	N/A
H2331				r	+	N/A

REPLACING FINGER-2 OR HEEL:

H2389				r	+++	+++
H2471				r	+++	+++
H2388				r	+++	+/-
H2410				r	+++	+++
H2429				r	+/-	N/A

CHANGING PATCHES OF RESIDUES:

H2381				r	+++	N/A
H2390				r	+	N/A
H2396				r	+	N/A
H2421				r	+/-	N/A

PAIRED CHANGES IN FINGER-2:

H2418				r	+++	++
H2420				r	++++	+/-

FIG. 9A

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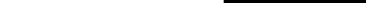
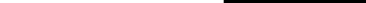
Attorney: Karen Mangasarian (Reg. No. 43,772)

For: **MODIFIED TGF- β SUPERFAMILY PROTEINS**

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OP-1 MUTANTS WITH C-TERMINAL ARGININE INSTEAD OF HISTIDINE:

H2247		+	+++
H2233		+	+++

BALANCING OF CHARGED RESIDUES IN FINGER-2 OF OP-1 MUTANTS:

Strain	Genotype	Phenotype	Notes
H2406	1,4,6,7 vktp r	+/-	N/A
H2443	1,4,6,7 25,26 vktp ed r	+++	++
H2447	1,4,6 25,26,30 ves ede r	+++	++
H2433	4 k r	+/-	N/A
H2456	4,6 25,26,30 es ede r	+++	+++

FIG. 9B

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CORRELATION OF REFOLDING EFFICIENCY AND CHARGED AMINO ACIDS
IN THE TGF- β (SEVEN CYSTEINE) DOMAIN

PROTEIN	FINGER-1	CXGXC	HEEL	FINGER-2	CXCX C-TERM	TOTAL OF CHARGED RESIDUES (+), (-) = TOTAL	NEGATIVE CHARGES, FINGER-2	NET CHARGES, FINGER-2	REFOLDING EFFICIENCY
OP-1	3+, 4-	2-	1+, 1-	4+, 2-	0	8+, 9- = 17	2-	2+	+/-
H2247	3+, 4-	2-	1+, 1-	4+, 2-	1+	9+, 9- = 18	2-	2+	+
H2447	3+, 4-	2-	1+, 1-	2+, 6-	1+	7+, 12- = 19	6-	4-	+++
BMP-3	4+, 4-	0	3+, 1-	3+, 4-	1+	11+, 9- = 20	4-	1-	+++
BMP-2	2+, 3-	1-	2+, 1-	2+, 6-	1+	7+, 11- = 18	6-	4-	+++
GDF-5	3+, 5-	1-	1+, 4-	2+, 4-	1+	6+, 14- = 20	4-	2-	+++
CDMP-2	3+, 5-	1-	1+, 3-	2+, 4-	1+	6+, 13- = 19	4-	2-	+++
GDNF	2+, 4-	0	6+, 4-	5+, 5-	0	13+, 13- = 26	5-	0	+++
TGF- β 1	5+, 3-	0	1+, 1-	5+, 2-	1+	11+, 6- = 17	2-	3+	+/-
TGF- β 2	5+, 3-	0	1+, 2-	4+, 3-	1+	10+, 8- = 18	3-	1+	+/-

FIG. 10

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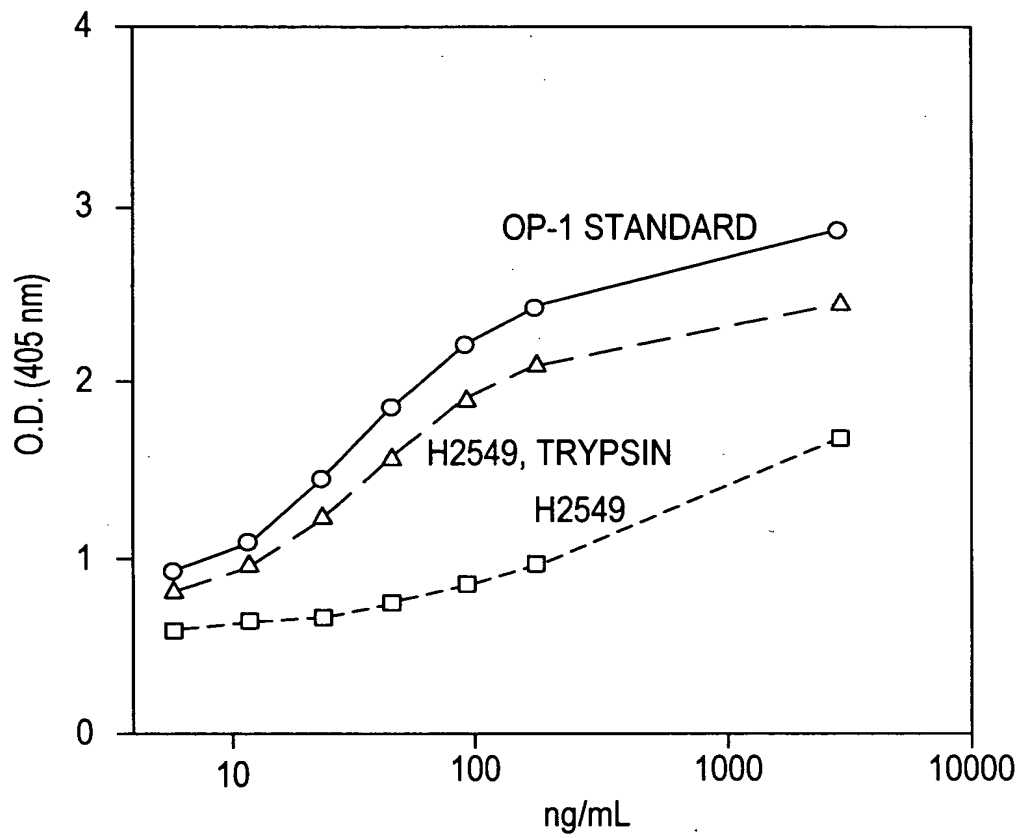


FIG. 11

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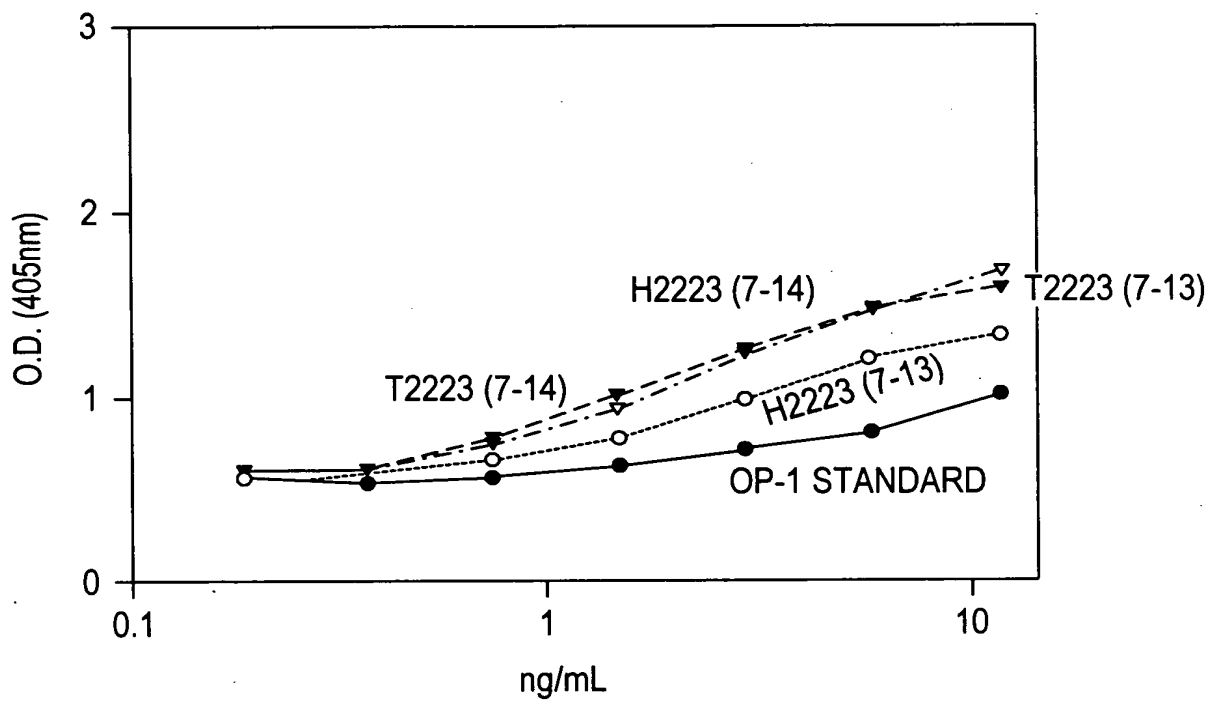


FIG. 12

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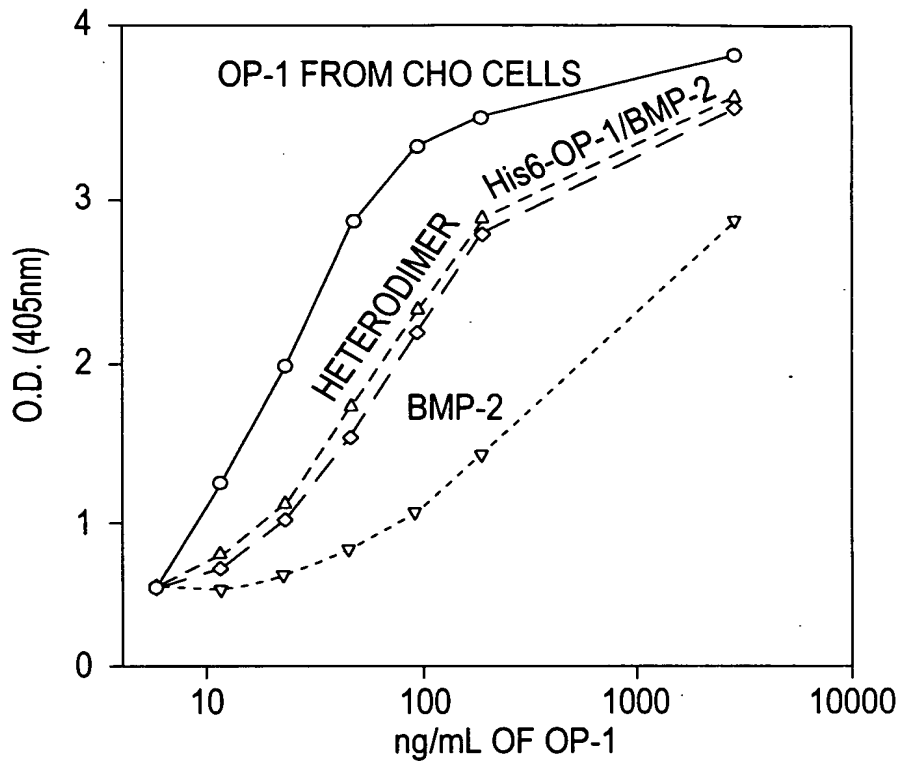


FIG. 13A

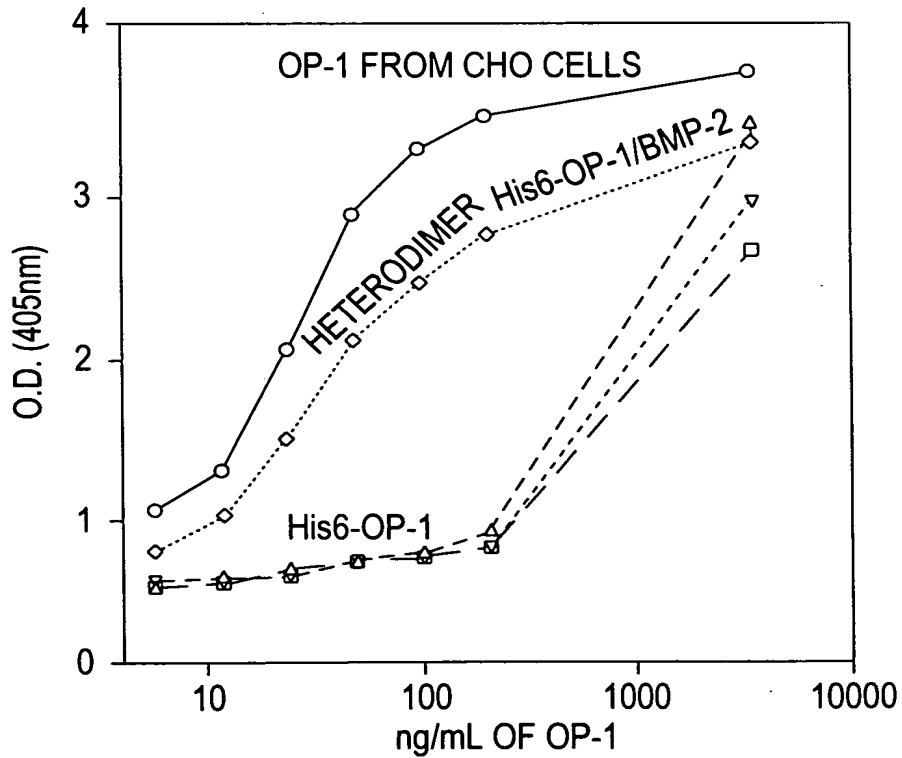
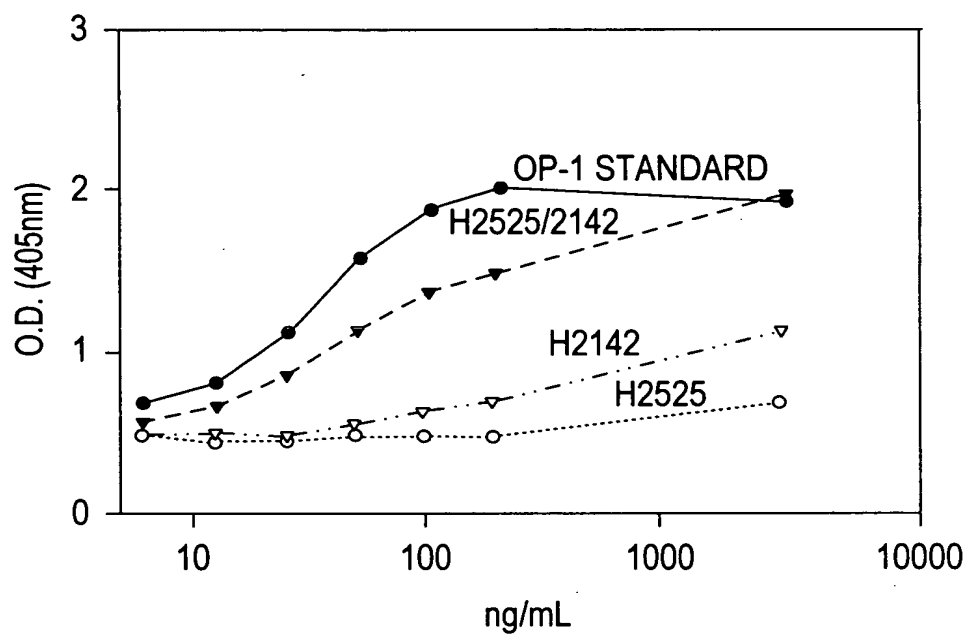


FIG. 13B

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**FIG. 14**

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CHANGES

FOLDING

ROS

FIG. 15

OP-1	K P C C A P T Q L N A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C H	-	5	[1]
2421	P T C C V P T R L S P I S I L F I D A S N N V V L K K Y R N M V V R A C G C R	(+)		
2406	N S C C V P T K L T P I S I L Y F D D S S N V I L K K Y R N M V V R A C G C R	(+)		[8]
2410	N S C C V P T E L S A I S M L Y L D E N E K V V L K N Y Q D M V V E G C G C R	3+	4	[18]
2247	K P C C A P T Q L N A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C R	(+)	3	[1]
2234	K P C C A P T Q L N A I S V L Y F D D S S N V I L K K Y E D M V V R A C G C R	(+)		[3]
2233	K P C C A P T Q L N A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	1+	3	[4]
2418	N S C C V P T K L T P I S V L Y F D D S S N V I L K K Y E D M V V E S C G C R	3+	2	[10]
2443	N S C C V P T K L T P I S V L Y F D D S S N V I L K K Y E D M V V R S C G C R	3+		[9]
2447	N S C C V P T E L S A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	3+	2	[9]
2457	N S C C V P T E L N A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	2+	3	[8]
2456	K P C C A P T E L S A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	3+	3	[6]
2460	K P C C A P T Q L S A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	3+		[5]
2457	N S C C V P T E L N A I S V L Y F D D S S N V I L K K Y E D M V V E A C G C R	2+	3	[8]
2449	K P C C A P T E L N A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C R	2+	3	[2]
2467	K P C C A P T E L S A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C R	(+)		[3]
2464	K P C C A P T Q L S A I S V L Y F D D S S N V I L K K Y R N M V V R A C G C R	(+)		[2]

FINGER-2 SEQUENCES OF OP-1 MUTANTS AND THEIR FOLDING EFFICIENCIES AND BIOLOGICAL ACTIVITIES IN THE ROS CELL BASED ALKALINE PHOSPHATASE ASSAY.

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